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SKIN IMPULSES AND LOCOMOTION IN *OIKOPLEURA* (TUNICATA: LARVACEA)

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Larvaceans are small pelagic tunicates which live in gelatinous houses that they secrete, drawing a feeding current of water through the house by oscillating the tail. Their anatomy and histology have been studied by a number of workers (*e.g.* Fol, 1872; Seeliger, 1893; Lohmann, 1933; and Martini, 1909). Although special methods for nerves were not used by these workers, the living animals are very transparent, so that it is possible to see some sensory structure in life, as well as the motor innervation of the caudal muscle cells. The organization of the nervous and muscular systems was therefore fairly accurately described long ago; it is seen diagrammatically in Figures 1 and 2.

An anterior "brain" containing a statocyst lies dorsally in the trunk region, from it nerves run to the lips and to the pair of ciliated stigmata through which the feeding current passes (Galt and Mackie, 1971). A dorsal nerve cord running over the stomach joins a caudal ganglion near the base of the tail; from the caudal ganglion a caudal nerve passes down the tail beside the notochord. During development, the tail rotates to lie in the horizonal plane, but the caudal nerve is morphologically dorsal to the notochord, as seen in Figure 2.

In the nerve cord of the tail there are conspicuous nerve cell bodies (Fig. 2), often paired and segmental in arrangement, reflecting the segmental origin of the motor axons, which terminate upon the inner surface of the caudal muscle cells in beautiful corymbiform motor endplates.

Other finer fibers (figured by Fol, 1872) also pass segmentally out of the nerve cord and terminate in branching, sometimes beaded, endings on the inner surface of the muscle fibers. Martini (1909) suggested that they were sensory terminations; it is, however, probable that these axons too are motor.

A pair of nerves (the *nervi recurrentes caudae* of Langerhans) pass forward from the caudal ganglion across the dorsal and ventral haemocoel of the tail of the
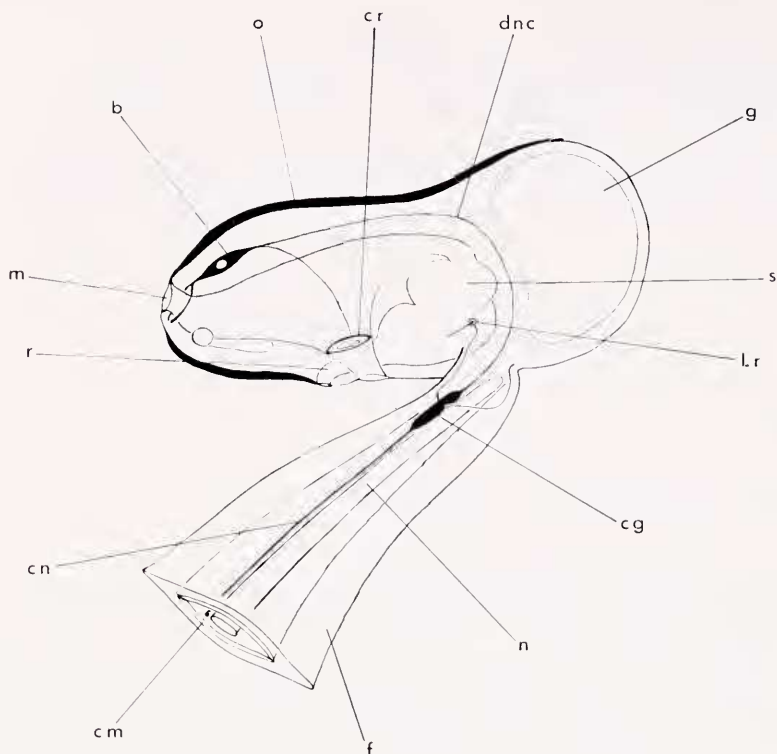
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FIGURE 1. Diagram showing organization of trunk and anterior tail region in *Oikopleura*. The tail has been twisted for clarity. b represents brain; cg, caudal ganglion; cn, caudal nerve; cm, caudal muscle cells; cr, ciliary ring; dnc, dorsal nerve cord; f, fin; g, gonad; Lr, Langerhans receptor; m, mouth; n, notochord; o, oikoplastic epithelium; r, rectum; and s, stomach.

paired bristle-bearing receptor cells on either side of the trunk which were discovered by Langerhans (1877). Seeliger (1893) suggested that these cells were sensitive to vibration. Apart from the receptor cells of the lips, the Langerhans receptor is the only exteroreceptor which has been observed in *Oikopleura*.

The muscular system operating the tail is composed of a small number of muscle cells (8–12 depending upon the species) lying on either side of the notochord and extending caudally beyond the notochord tip. These cells interdigitate with adjacent cells in a characteristic way (Bogoraze and Tuzet, 1969) and have an inner myofibrillar zone and an outer mitochondrial zone. They are focally innervated by the corymbiform endplates, and transmission is apparently cholinergic (Flood, 1973).

The outer surface of the animal is not covered by a tunic, as in other tunicates, but is composed of an exposed epithelial layer consisting of thin polygonal cells; in the oikoplastic regions of the trunk the cells are thicker and highly specialized for secretion of the house.

Although the structure of *Oikopleura*, summarized briefly above, is fairly well known, little is known of the physiology of the animal. In a previous paper (Galt

and Mackie, 1971) the electrical correlates of ciliary reversal were investigated. It was also found that small potentials could be recorded from the surface of the trunk when the epithelium was touched; these events were suggested to be propagated skin impulses.

In the present paper we show that the epithelium covering the animal is indeed capable of propagating skin impulses, and that these are linked to the neurons driving the locomotor rhythms of the animal.

MATERIAL AND METHODS

Two species of *Oikopleura* were invesigated. Unless otherwise stated, the species referred to in this paper is the large *Oikopleura labradoriensis* Lohmann, which was common in the plankton off the Friday Harbor laboratory throughout April 1974. Large specimens of this species had tails some 7 mm long. By June it had become scarce, and the smaller *O. dioica* Fol was used for some experiments. The animals were dipped from the water off the laboratory dock, and transferred
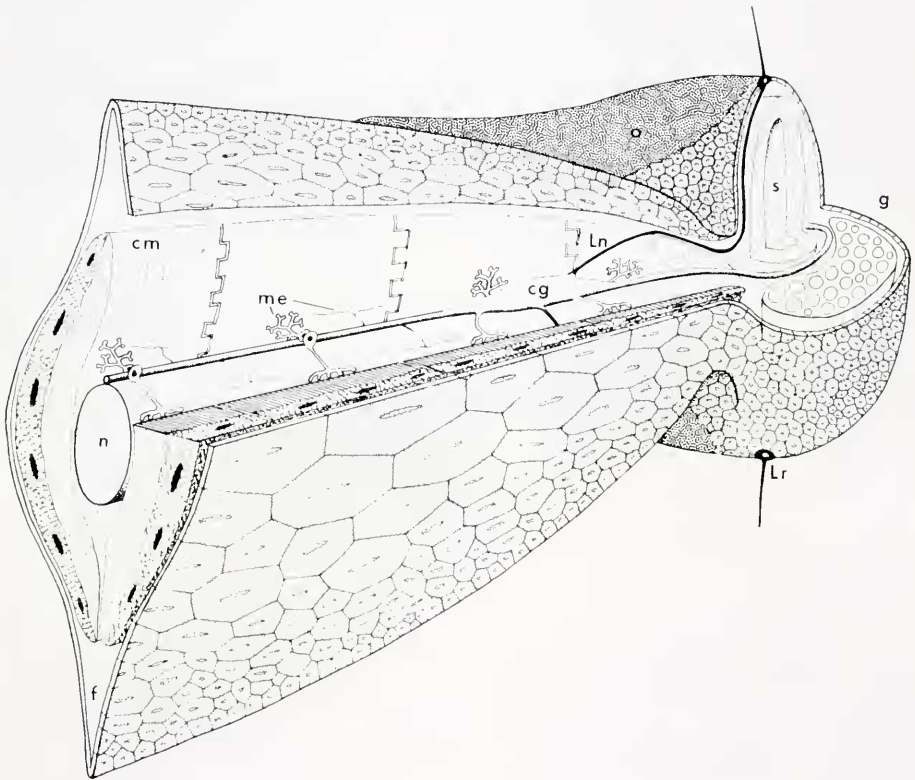

FIGURE 2. Stereogram of anterior caudal region. The oikoplastic epithelium of the trunk region is shaded. Note the Langerhans nerves arising from the caudal ganglion and passing to bristle-bearing receptor cells, and two types of motor ending. cg represents caudal ganglion; cm, caudal muscle cell; f, fin; g, gonad; Ln, Langerhans nerve; Lr, Langerhans receptor cell; me, motor endings; n, notochord; and o, oikoplastic epithelium.
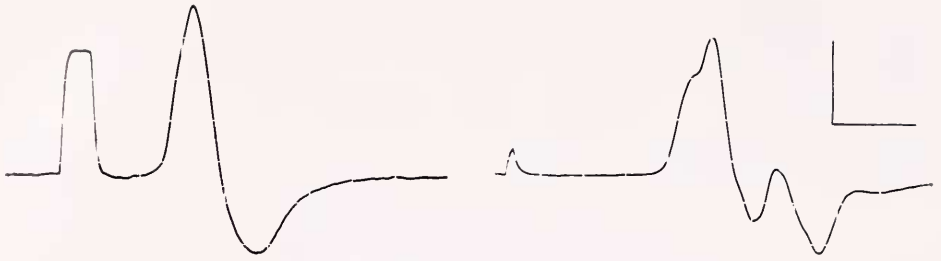
FIGURE 3.   Skin impulses evoked by electrical stimulation of isolated fin
region; scale bars: 500 μv, 4 msec.

to small aquaria in the laboratory for observation of their behavior while still in
their houses.

Electrical recordings were made from specimens held by suction electrodes or
pinned down on Sylgard platforms with cactus spines. The recording dish was
mounted on a Peltier cooling ring.

Plastic suction electrodes were used for external recording. Sea water was
drawn up into the tube, and the animal was held to the tip by suction. Such
electrodes were also used for stimulation. Input was fed to a Grass 79C poly-
graph with EEG amplifiers, and the amplified signal was displayed concurrently
on a Tektronix 5103N storage oscilloscope. Stimulating shocks of 1–2 milli-
seconds duration were given with a near-threshold voltage setting.

In some experiments, input from the suction electrodes was fed to a Gould
Brush 220 oscillograph, via the amplifiers of a Tektronix 502 oscilloscope.

Living and Flemming-fixed specimens were examined by Nomarski interference
microscopy; material for electron microscopy was fixed in 2.5% glutaraldehyde
in Millonig's phosphate buffer, followed by postfixation in 1% osmium tetroxide.
Sections were stained with uranyl acetate and lead citrate and examined in a
Philips EM 300.

RESULTS

Skin impulses

When suction electrodes are attached to any part of the surface of the tail or
posterior trunk region, and the skin is stimulated electrically or mechanically, it
is possible to record impulses which spread across the surface from the point
stimulated. These are large events, (1–2 millivolts), with an initial positive going
component; the form of the pulse being rather variable (Fig. 3a and b). By
placing a stimulating electrode on the tail tip, and two recording electrodes at dif-
ferent distances away from it, records such as Figure 4 are obtained. Within the
areas in which they are conducted, the skin impulses show non-decremental all-or-
none spread. Conduction velocities lie within the range 15–21 cm/sec at 12–13° C,
with an average of 17.8 cm/sec for a number of records from different specimens.

These values refer to the first impulses obtained from preparations which had not been stimulated in the recent past. Conduction velocity decreases with repeated stimulation, the rate of decline being related to frequency of stimulation as shown in Figure 5. Measurements from preparations where the impulse passes successively beneath two recording electrodes show that the increase in conduction time represents a decrease in conduction velocity, not an increased initiation lag.

The impulses spread diffusely in all directions. They can pass around the edge of the tail fin and invade the contralateral side. They spread to (and can be evoked from) the epithelium covering the gonad and hinder trunk region, but are not found in the specialized oikoplastic epithelium of the anterior trunk region (Figures 1 and 2). Stimulation in this region does not yield a behavioral response except near the lips, where sensory cells are located. Lip stimulation affects the ciliary beat of the stigmata, the response being mediated by a nervous pathway (Galt and Mackie, 1971).

We have not succeeded in making intracellular records from the single layer of epithelial cells some 3 μm thick, which forms the skin of the animal, but there is compelling evidence that the skin impulses are in fact conducted by these cells.

The impulses spread diffusely across the surface of the animal, and in the regions where they spread, there is a uniform degree of excitability, so far as we can determine. Interruption of the epithelial sheet by abrading the skin around the tail blocks conduction across the damaged belt. On the other hand, deep incisions into the tail, including cuts which sever the nerve cord, do not block conduction provided that a skin bridge is left intact. The impulses can be recorded
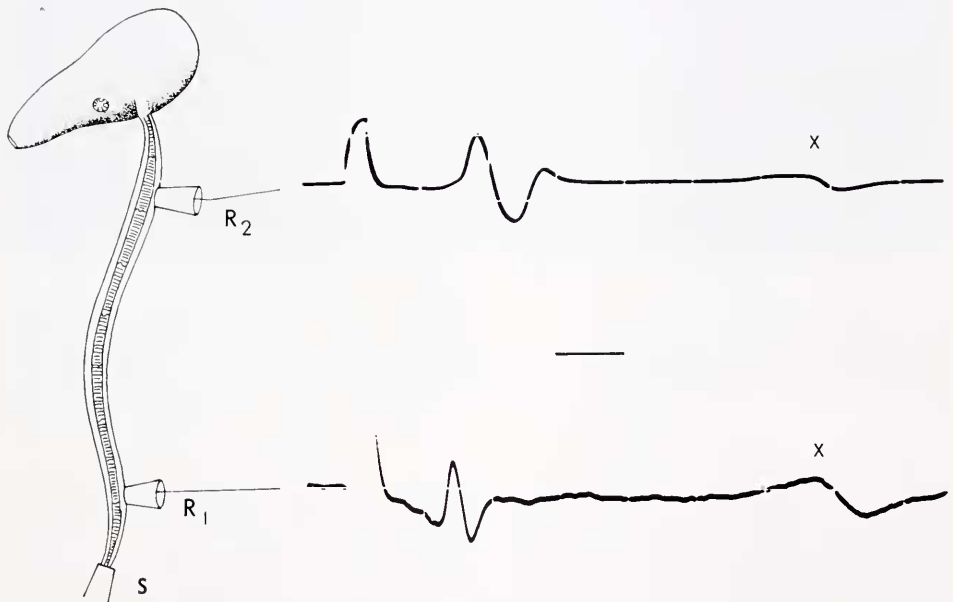

FIGURE 4. Propagation of skin impulses. The position of stimulating (S) and recording electrodes (R₁, R₂) are shown at left. The cross marks the beginning of tail movement evoked by the skin impulse.

from isolated strips of the tail fin, which consists essentially of a simple outfolding of the skin. Nerve fibers in the tail can easily be followed in the living animal to their terminations associated with the inner face of the muscle bands.

If there was a general innervation of the skin, or a nerve net underlying it, this should be readily visible, but no such elements have been seen, either in the living animal, or in numerous EM sections of the skin in the two species examined. The only nerves which reach the skin in the areas we are concerned with are those which go to the two lateral bristles on the posterior ventral trunk wall, which, as we show below, provide the pathway whereby skin impulses reach the central nervous system. Fol (1872) described (in another species) a caudal receptor cell and fiber lying at the extreme tip of the tail; in the two species we have examined, a cell with a long process is found at the tip of the tail, but it does not seem to be in connection with the nerve cord, and is probably a connective tissue cell.

The epithelial cells of the skin are connected by gap junctions (see below) which offer a potentiality for electrical, ionic, and metabolic communication (Gilula, Reeves and Steinbach, 1972). Conduction across an electrically-coupled sheet of cells would not be suppressed by drugs which block cholinergic synapses. We find that skin conduction continues in preparations treated with levels of Mg^{++} which suppress muscle rhythms, presumably by blocking the nervous pathways through which the muscle is activated.

Skin impulses having many features in common with those described above have been demonstrated in hydrozoa and in amphibian larvae. In the latter, intracellular records have confirmed that the events originate within epithelial cells. We have also found that skin impulses are present in the tadpole larvae of the ascidian *Dendrodoa grossularia,* and have been able to record these intracellularly from the epithelial cells of the tail (Mackie and Bone, in preparation). Finally, skin impulses are not found in the larvacean *Fritillaria pellucida,* and histological examination shows that epithelial cells are absent in this species (Bone, Fenaux and Mackie, in preparation).
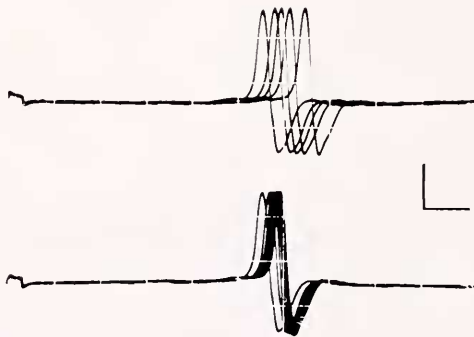

FIGURE 5. Decline in conduction velocity of successive skin impulses evoked by electrical stimulation. The upper is stimulated at 0.5/sec; the lower is stimulated at 1.5/sec; scale bars: 500 μv; 5 msec.
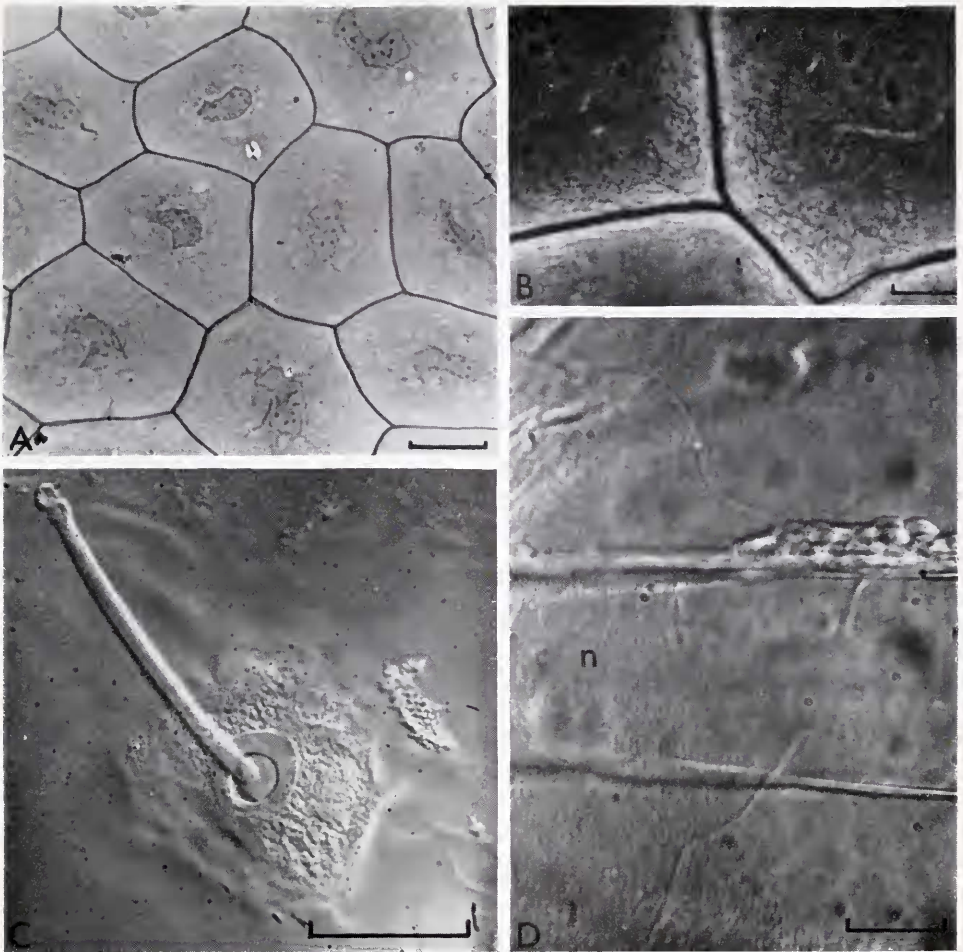
FIGURE 6. a) Epithelial cells covering gonad region fixed in Flemming without acetic and stained with iron haematoxylin; b) the same region as (a) at a higher magnification showing the fibrous appearance of the cytoplasm; c) the Langerhans receptor cell; in this preparation, which was fixed in Flemming without acetic and examined with Nomarski interference contrast, the tip of the bristle has been damaged; d) The caudal ganglion and Langerhans nerves seen in the living animal with Nomarski intereference contrast. The junction of the tail with the trunk is to the left of the figure; n indicates the notochord.

These considerations leave little doubt that the skin impulses of *Oikopleura* are propagated across the epithelial cell sheet which forms the outer surface of the animal. The wave forms recorded with suction electrodes show considerable variation. In their simplest form, skin impulses are smooth biphasic events, but secondary blips, shoulders and composite wave forms are also seen. It must be borne in mind that suction electrodes vary in bore and resistance; in the amount of tissue they cover or imbibe (partly dependent upon the suction applied); in the extent to which they damage the cells under or around the point of attachment;

and the extent to which they may leak under the stress of sudden movement. Some distortion of what is assumed to be a conventional waveform is inevitably to be expected in recordings made with this technique. Distortion resulting from capacity-coupling in the amplifiers is probably slight, since records made with d-c amplifiers differ little from those (figured in this paper) using a-c coupled amplifiers.

In our records, the typical skin impulse has a duration of 8–12 msec. There is a correspondingly short refractory period of some 5 msec.

Little or no attenuation of the second impulse occurs with pulse pairs 12 msec apart, again suggesting that the membrane is fully repolarized within that period.

Impulses propagated in the skin pass to the locomotor centers in the central nervous system and either evoke a swimming burst, or accelerate existing swimming activity, as we discuss in a separate section below. In fresh specimens there is a one-to-one coupling between skin pulses and initial muscle response. There are no cilia on the outside of the animal nor secretory cells in the conducting regions, so that in the absence of such local skin effectors, there is no reason to suppose that skin impulses act other than as part of the afferent sensory pathway in the locomotory escape response.

Structure of the epithelial cells of the skin

The cells which propagate the skin impulses are large flattened polygonal cells (Fig. 6, a and b) whose borders interdigitate slightly.

Larger cells cover the lateral surface of the tail than elsewhere (Fig. 2). The largest are around 250 μm across, elongated in the anterioposterior direction, as are their spindle shaped nuclei. The ultrastructure of these cells is striking, for they are divided into two distinct layers; an outer fibrous or "cuticular" layer without mitochondria or other cell organelles, and a thin inner layer containing the nucleus, mitochondria, Golgi appartus, and rough ER (Fig. 7). It is possible that the fibrous layer of the epithelial cells is made up of tunicin fibrils, since in size and arrangement, the fibers resemble those in the test of other tunicate groups. On the outer surface of the cell is an irregularly beaded layer. This peculiar arrangement suggests that the epithelial cells are divided functionally into an outer "cuticular" zone, and an inner zone in which the vital functions of the cell take place. As we shall see in a later section, *Oikopleura* is remarkably impermeable to drugs if the epithelial layer is intact. Unlike other tunicates, the adult *Oikopleura* does not possess a tunic, for although present in the larva, the tunic ruptures and is lost as the animal increases in size (Galt, 1972). This unusual outer layer of the epithelial cells is not present in the ascidian tadpole larva (Mackie and Bone, in preparation), which is equally impermeable to drugs, but which *does* possess a tunic. Adjacent epithelial cell membranes are separated by a gap of some 200 Å (Fig. 7), except for a limited region near the bases of the cells where the two membranes are more closely apposed forming gap junctions. Since these gap junctions are not visible in every section of two adjacent cells, it appears that they do not form a continuous band around the bases of the cells, rather that they are distributed in a punctate manner.
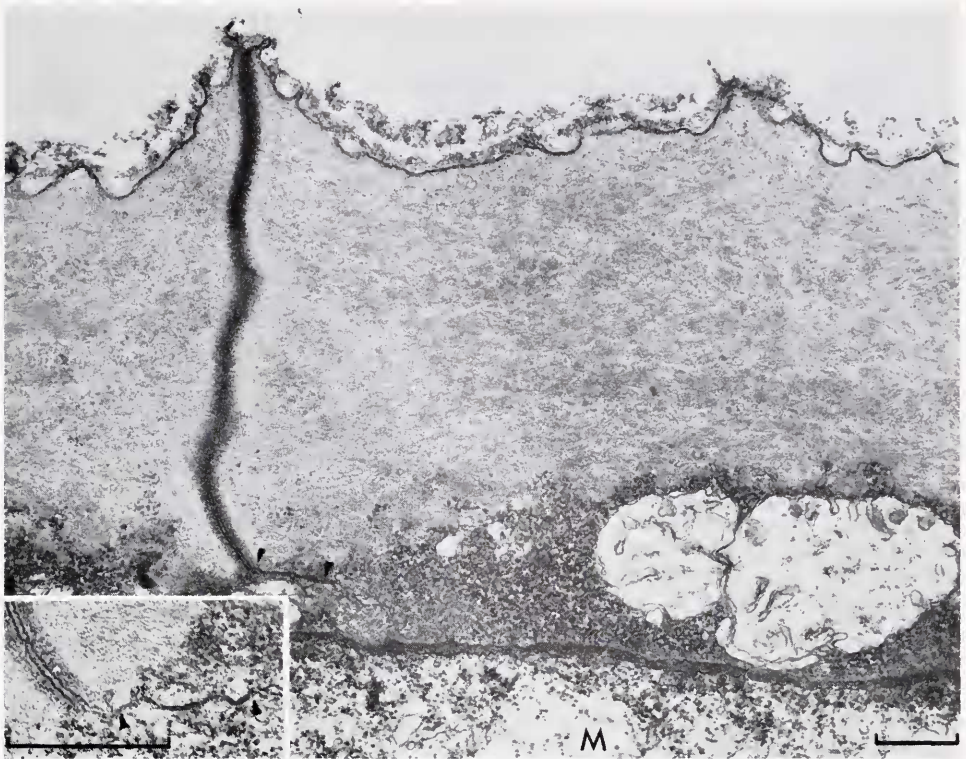
Figure 7. Junction between two epithelial cells of trunk. Note outer "cuticular" region of epithelial cell, and inner mitochondrial zone. The adjacent cell membranes lie 200 Å apart (inset) except for a gap junction (arrowed) near the inner face of the cells. M represents underlying muscle cell; scale bar 0.5 μm.

Motor patterns of the tail

The activity of the caudal musculature in *Oikopleura* (studied in *O. dioica* by Galt, 1972) is essentially rhythmic, varying from long cycles of oscillation to single beats at intervals; usually short bursts of tail movement alternate in a regular way with periods of inactivity (Fig. 8). Both amplitude and frequency of the tail movements are variable, so also are the lengths of the bursts of activity and the interburst periods. Our records of these patterns of activity were obtained by attaching suction electrodes to the trunk or tail; potentials serving to indicate tail movements arise as the animal moves its tail, but it is not certain whether these are movement artifacts or whether at least in part, they represent electrical activity of caudal muscle cells, as Galt and Mackie (1971) previously suggested.

Glass microelectrodes placed with their tips in the caudal muscle cells of animals whose tail movements have been restricted by pinning down the tail, provide rather similar records (compare Fig. 8 a and b), but similar difficulties of interpretation arise. It seems on the whole most probable that the potentials recorded by suction electrodes are extracellularly recorded muscle potentials.
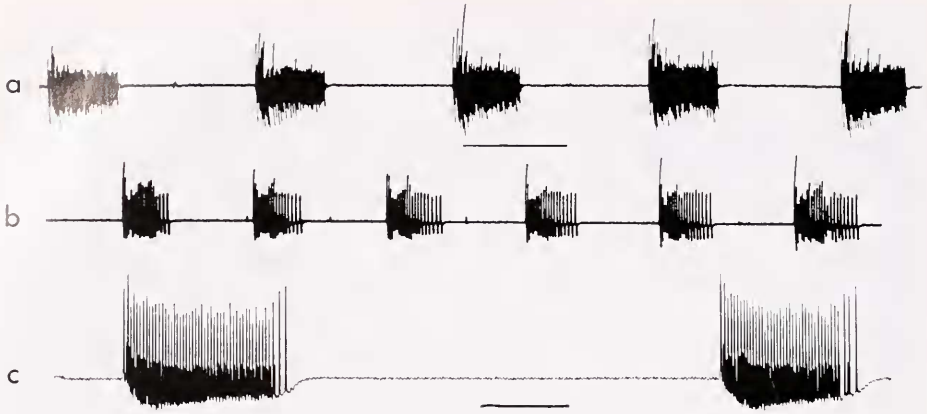
FIGURE 8. Swimming activity: (a) suction electrode record; (b) record obtained from microelectrode (in caudal muscle cell); (c) suction electrode record (*O. dioica*); scale bars: a and b: 10 sec; c: 1 sec.

Three general patterns of spontaneous activity can be distinguished in intact animals: pumping behavior in the house; swimming behavior; and house rudiment expansion behavior.

Pumping behavior. When occupying the house in which it feeds, *Oikopleura* pumps water through the house (thus moving the house forward through the
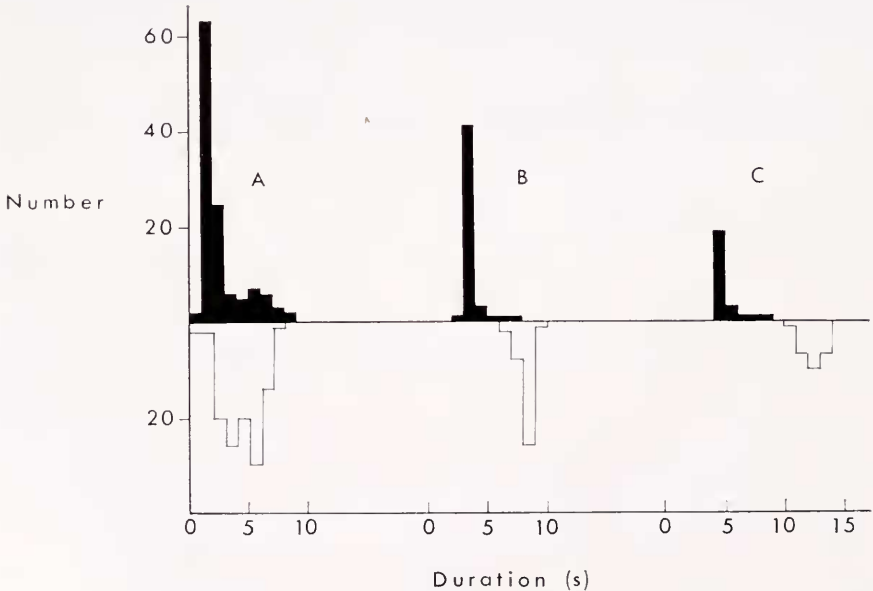

FIGURE 9. Length of burst (filled) and interburst (open) periods during periods of: (a) pumping behavior in the house; (b) swimming behavior of intact animal attached to electrodes; and (c) isolated tail attached to electrodes.

water) by rhythmic bursts of oscillation of the tail at frequencies between 2.5 and 3.0/sec. This activity is cyclical; in an animal observed for 25 min in an aquarium, the length of the bursts of tail activity varied somewhat, as did the interburst intervals, but over short periods, both are very regular (Fig. 9). These low frequency bursts characteristic of pumping behavior within the house, may be shown for a few minutes after animals have been removed from the house and attached to suction electrodes, but more usually, although burst length and inter-burst interval remain similar, the frequency of oscillation within bursts increases almost at once, and a different pattern of activity appears.

Swimming behavior. When feeding in the house, *Oikopleura* is very sensitive to vibration, even light touch of the house causing the animal to leave in a burst of swimming at relatively high frequency. This is then followed by a similar rhythm of activity to that of pumping behavior, but at higher frequency, usually between 5 and 6 c/sec within the bursts of activity. The animal swims upwards in the water column, and sinks vertically head downwards during the periods of inactivity. The timing of bursts and interburst periods in animals swimming freely in aquaria is similar to that shown when they are attached to suction electrodes; for a given animal over periods of 15–20 min or so, this pattern of activity is remarkably regular (Fig. 9b). Over longer periods, the pattern may change; it is slightly different between different animals. During the swimming burst, the frequency of tail oscillation usually remains the same (see Fig. 12).

Removal of the anterior part of the trunk (containing the cerebral ganglion), or separation of the tail from the trunk by cutting across the base of the tail, does not significantly alter the swimming pattern; the isolated tail (Fig. 9c) shows a similar pattern to the intact animal. Successive removal of portions of an isolated tail beginning at the tail tip does not abolish the swimming rhythm even if only 2 muscle cells remain on either side of the tail stub, but if the tail is progressively shortened in a similar way, beginning at the base, then spontaneous activity ceases as soon as that portion of the tail containing the caudal ganglion has been removed. Isolated tails which have been cut across so as to remove the caudal ganglion not only do not exhibit spontaneous activity, but respond to high levels of electrical stimulation with single beats only, and rhythmic activity cannot be
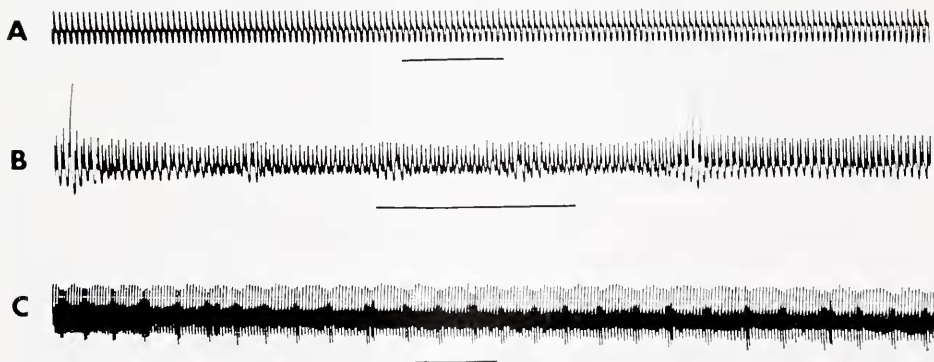

FIGURE 10. House rudiment expansion behavior. Note larger rhythm superimposed on steady oscillation in B and C; scale bars: A, 10 sec; B, 5 sec; C, 15 sec.

evoked. Stimulation must be strong to excite such preparations, the muscle cells are presumably stimulated directly.

We conclude therefore that swimming behavior is dependent upon the presence of the caudal ganglion, and that input from the cerebral ganglion is not required for rhythmic swimming. This conclusion is supported by experiments in which the caudal ganglion is hemisected by transecting the tail at the level of the ganglion, or is interfered with mechanically by touching with a microelectrode. Experiments of this kind yielded preparations which either did not show any spontaneous activity or which showed single tail beats at 5 sec or 10 sec intervals. In one case, irregular tail beats at intervals of 1–12 sec appeared, with occasional bursts of up to 5–6 beats.

Such patterns of activity after partial destruction of the caudal ganglion are also produced by the action of various blocking agents upon the intact animal. *Oikopleura* is remarkably resistant to drugs such as curare, procaine and atropine, and also to high concentrations (up to 100 mM/lr) of Mg^{++}, Mn^{++} and Co^{++}. For example, animals will swim for 30 min or longer in equal parts of sea water and isotonic magnesium chloride solution. Evidently, the external epithelium is extremely impermeable (as its structure would suggest), and the situation is similar to that found for the ascidian heart by Kriebel (1968), where drugs such as acetylcholine and adrenalin are ineffective unless allowed access to the *inner* wall of the heart. When drug access to the muscles and nervous system of *Oikopleura* is allowed by cuts in the epithelial wall, curare and Mg^{++} reversibly block rhythmic activity, and procaine does so irreversibly. Before activity ceases, single tail beats at 5 sec or 10 sec intervals are produced by all three drugs.

Lower concentrations of curare (below 10^{-5}) sometimes induce a rhythm of single beats at 2 sec intervals, without abolishing bursts of swimming activity.

Our experiments were usually carried out between 12 and 13° C; in four experiments the effects of varying the temperature of the bath in which the animals lay were investigated. Swimming activity is not abolished by lowering the temperature until the bath freezes: above 17° C however, the animals begin to swim erratically and soon die.

Change in temperature between 4° C and 16° C does not significantly alter the frequency of oscillation of the tail during bursts of activity although heart rate, and the metachronal rhythm of the ciliary rings declines as the temperature falls. Interestingly enough, the frequency of ciliary reversal pulses (Galt and Mackie, 1971) is more or less linearly related to temperature.

House rudiment expansion behavior. This pattern of long bursts (up to 4 min) of tail oscillation at frequencies around 4–5/sec is shown by animals which are expanding the house rudiment secreted by the oikoplasts of the trunk (Fig. 10). It was first described in *O. dioica* by Galt (1972), who observed that in animals free to move, house rudiment expansion behavior (REB) resulted in a "nodding" of the trunk which presumably serves to free the rudiment from the underlying oikoplasts, and enables it to be expanded. If the house rudiment is removed from an animal showing the almost continuous activity of REB, or if the animal itself shakes off the rudiment, REB stops and swimming behavior begins. REB is not shown by isolated tails, nor by animals in which the lips have been cut away (damaging the oikoplasts as well as resulting in removal of
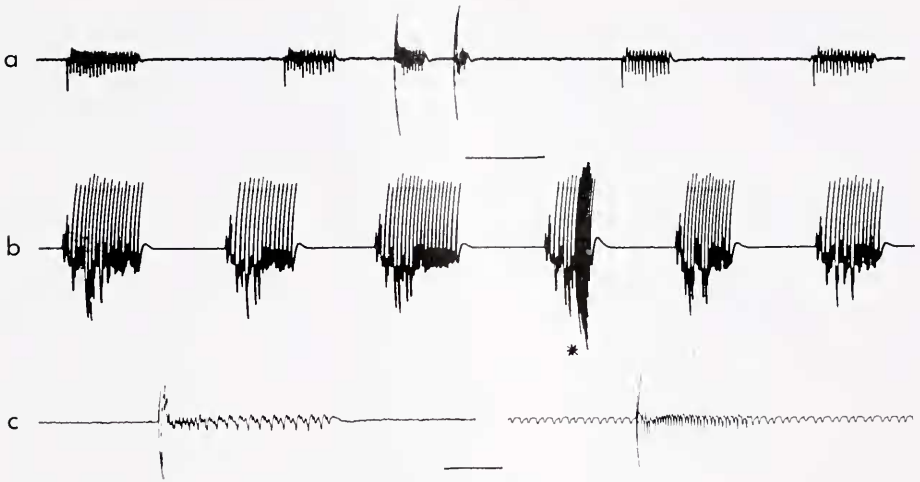
FIGURE 11. a) Skin impulses evoked by electrical stimuli reset the locomotor rhythm. Note that after the second skin impulse (the large potentials), a short swimming burst is evoked, and the interburst period after this is similar to that before the skin pulses; b) a skin impulse evoked by electrical stimulation (at the asterisk) accelerates the locomotor rhythm; c) two skin impulses evoked by electrical stimulation of the same animal. During an interburst period, the skin impulse elicits a burst of swimming, whereas during a period of steady oscillatory activity, the skin impulse accelerates the existing rhythm. The scale bars for the upper pair of records: 5 sec; for the two isolated skin impulses below: 1 sec.

the cerebral ganglion). It seems that this pattern of behavior is dependent upon input to the caudal ganglion from the cerebral ganglion.

Superimposed upon the 4–5/sec oscillations during REB are often larger rhythms marked by increased amplitude oscillations with little change in frequency (Fig. 10, B and C).

These are usually at intervals of 5–10 sec and may appear as gradual amplitude increases during the usual pattern of oscillation of REB, or may be single beats only of larger amplitude. It is striking that the larger rhythms superimposed upon the usual REB rhythm are of similar frequency to the rhythms observed when the caudal ganglion is partially destroyed, or when drugs are applied to the animal.

Resetting the motor rhythms. As noted above, skin impulses initiate movement in quiescent animals or increase the frequency of movement in animals which are swimming or expanding a house (Fig. 11). When animals are swimming in regular bursts, the interval between such an induced burst of activity and the succeeding spontaneous burst is the same as that between spontaneous bursts (Figs. 11a, 13a) so that the effect of stimulation is to reset the rhythmic pattern of activity, whether or not the induced burst of activity is shorter than preceding or succeeding bursts. The initial frequency of tail movement following stimulation is invariably much higher than that in spontaneous bursts, and declines during the burst in a linear manner (Fig. 12). Stimulation during REB may either result in a frequency increase, followed by a decline to a frequency below the characteristic 4–5/sec before the original frequency of oscillation is regained, or, rarely
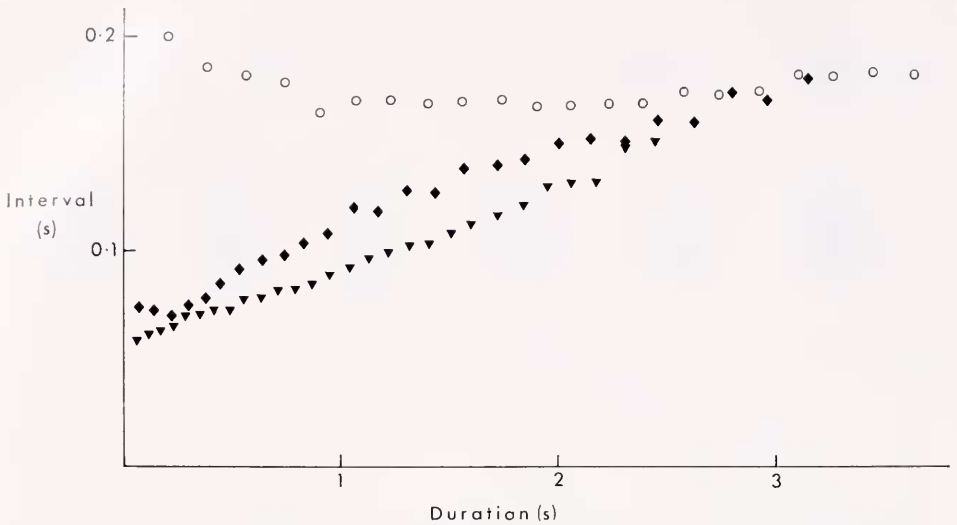
FIGURE 12. The frequency of swimming beat in a spontaneous swimming burst (open circles) compared with that in two bursts evoked by skin impulses (filled diamonds and triangles). The ordinate is the interval between successive tail movements; abscissa, the duration of the burst.

there may be no frequency increase, and the burst of REB activity stops shortly after the stimulus.

The afferent pathway from the skin. Since skin pulses evoke movements or accelerate movements in intact animals, and in animals which have had the anterior
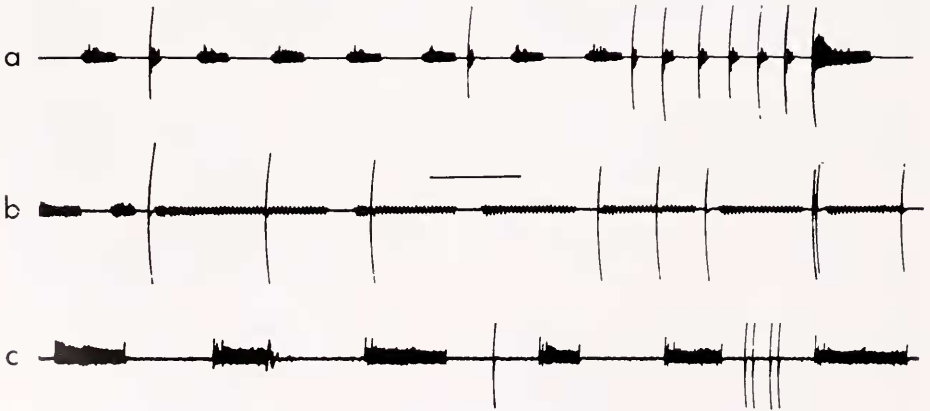

FIGURE 13. a) In the intact animal, skin impulses (large potentials) reset the loco-motor rhythm; b) in the isolated tail, skin impulses do not affect the locomotor rhythm; c) after bi-lateral section of the Langerhans axons in an otherwise intact animal, skin impulses no longer reset the locomotor rhythm. In this record, the upper line indicates the stimulus and the large potentials on the lower line are skin potentials. For all records, the scale bar is 10 sec.

part of the trunk removed, but do not do so in isolated tails (Fig. 13b), it is evident that the link between the skin pulse and the central nervous system must lie in the posterior region of the trunk. An obvious route linking the skin with the caudal ganglion is provided by the paired bristle bearing cells lying on either side of the posterior trunk region, which are connected with the caudal ganglion by the nerves of Langerhans (Langerhans, 1877). Fortunately these nerves (actually single axons, 5 microns in diameter) are visible in the intact animal (Fig. 6d), so that it is possible to transect them to determine the effects of this operation on the transmission of stimuli to the cns. When recording electrodes are placed on mid-tail and on the trunk region, and a stimulating electrode is placed on the tail, it is found that unilateral section of the Langerhans axon does not block conduction between the skin and the caudal ganglion, whereas after bilateral section, although skin pulses pass from tail to trunk, they no longer evoke tail movements (Fig. 13c). In control specimens with incisions of equal severity which left a Langerhans nerve intact, conduction peristed.

It is clear, therefore, that one function of the Langerhans bristle-bearing cells and the Langerhans axons, is to provide a route connecting the skin pulses with the caudal ganglion controlling the motor activity of the tail. It is not clear, however, whether the bristle-bearing cells themselves (assumed on morphological grounds to be sensory receptors) can initiate impulses in the Langerhans axons which will affect motor activity. Seeliger (1893) suggested that the bristles were embedded in the material of the house, and provided sensory information to the tail, perhaps sensing vibration of the house and triggering the animal's flight reaction (escape from house). We have made numerous attempts to alter the rhythmic activity of the tail by bending the bristles of these cells with micro needles. The bristles are relatively stiff, so that it is possible either to touch them near to the tip (when the basal region remains more or less static while the tip springs back from its bent position), or to bend the entire bristle by touching it near the base.

On one occasion only did we succeed in altering the rhythm of regular tail activity without inducing a skin pulse. On many other occasions, although skin pulses and tail movements could be evoked by touching the epithelium near the base of the bristle-bearing cell, touching the bristle itself gave no response.

It is difficult to assess such experiments, for although repeated trials (both with *O. labradoriensis* and *O. dioica*) gave negative results, it is possible that the incorrect stimulus was applied, or that the bristle had been damaged in some way—in the house the system may work as Seeliger (1893) suggested. However, since animals are often found in which the bristles are damaged (Fig. 6c), and since they are relatively much larger in juveniles (Galt, 1972), it is perhaps possible that in the adult animal it is no longer functional as a vibration or mechanoreceptor.

The coupling between skin pulses and the motor response, *via* the Langerhans receptor and caudal ganglion, is very close in fresh specimens so that each skin pulse evokes a motor response. In preparations which have been stimulated to yield many successive pulses, or in preparations treated with Mg^{++}, the skin pulses and the motor response may be uncoupled before Mg^{++} abolishes the motor response. Preliminary ultrastructural investigations of the Langerhans receptor

suggest that the Langerhans nerves are axons of cells lying in the caudal ganglion, which synapse with the bristle bearing receptor cells themselves; persumably it is at this synapse that the system is uncoupled.

DISCUSSION

The observations reported above confirm the existence of propagated skin impulses in *Oikopleura,* providing another example of the same type of phenomenon known in siphonophores and hydromedusae (Mackie, 1965; 1970; Mackie and Passano, 1968), and in amphibian larvae (Wintrebert, 1904; Roberts, 1969; 1971; Roberts and Stirling, 1971).

In the hydromedusan *Sarsia,* a conducting epithelium such as the subumbrellar endoderm lamella conducts all-or-none impulses at velocities of 20–25 cm/sec (at 18° C). The wave forms recorded extracellularly are typically biphasic, with an initial positive component around 1 mv, and a refractory period of about 12 msec.

The corresponding values for *Oikopleura* skin are 15–21 cm/sec (at 12–13° C), 1–2 mv, and 8–12 msec. In *Sarsia,* the conducting layer functions as part of the pathway whereby impulses from the excitable exumbrella epithelium reach the subumbrellar muscles whose contraction brings about a protective involution of the bell known as "crumpling." In *Oikopleura,* as in the amphibian larvae, the excitable skin acts as a part of the afferent sensory pathway by which stimulation of the external surface evokes impulses which travel to the central nervous system and trigger locomotory flight behavior.

It is a characteristic of epithelial conducting systems that they function in situations where the animal has to respond quickly by flight or by a protective response, to stimuli which may impinge upon it anywhere over a wide surface area. They provide a simple and effective (if crude) sensory adjunct to the nervous system. Conduction is diffuse and unpolarized, and no especial cells seem to be differentiated within the layer for conduction. The cells which conduct are the ordinary covering epithelial cells, and the enrollment of such cells into the response mechanism is manifestly efficient in terms of neuron economy.

In amphibians, the skin impulse system provides the tadpole with a precocious ability to respond to external stimuli (Wintrebert, 1904), being functional at a stage before the skin receives its definitive sensory innervation. During later development, this transitional device is lost, at different stages in different species (Roberts and Smyth, 1974). The skin impulses of amphibian larvae resemble those of *Sarsia* and *Oikopleura* in many respects, but conduction is somewhat slower (7.7 cm/sec in *Xenopus,* and 4.5 cm/sec in *Bufo*) and the impulses are of much longer duration, over 100 msec in *Xenopus* and up to 900 msec in *Bufo.* Roberts and Stirling (1971) suggest that this long duration is adaptive as it will set a long refractory period for the behavioral response, obviating useless flutter; no such consideration applies in the case of *Oikopleura* where the skin impulses are relatively very rapid events.

In *Xenopus,* the skin impulses are TTX-sensitive and appear to be generated across the inner membranes of the epithelial cells. The cells are electrically coupled, and impulses probably spread by direct current flow. Close appositions (gap junctions) are seen between adjacent cell membranes, presumably representing low resistance routes. Without intracellular recordings, it will not be

possible to establish unequivocally that electrical spread occurs between cells in *Oikopleura,* but the existence of gap junctions between cells, and the all-or-none character of the spread is compatible with this interpretation, which is strongly suggested by the considerations listed earlier in the results section.

Larvaceans are interesting because they more closely resemble the larval stages of other tunicates than they do other adult tunicates; the group is generally accepted to have arisen by paedogenesis. Thus the larval tail, notochord, and other features of the ascidian or doliolid larvae are retained (Garstang, 1928). We have recently found that skin conduction occurs in the ascidian tadpole (Mackie and Bone, in preparation), and at least one group of chordates (anuran Amphibia) show skin conduction in the larval stage; perhaps we are concerned with a larval adaptation of possible widespread occurrence which has persisted in the adult *Oiko-pleura* as part of its paedogenic syndrome. Embryonic cells are frequently electrically coupled (*e.g.,* Bennett and Trinkaus, 1970), so that in a sense, larvae may be preadapted to non-nervous impulse conduction, utilizing it to enconomize in neuron number either until development of the definitive sensory apparatus takes place (amphibia), or to compensate for the drastic reduction in numbers of all cell types apparently consequent upon the production of large numbers of larvae (tunicata).

Considering the relatively small numbers of neurons and muscle cells involved, *Oikopleura* has quite a wide repertoire of patterns of tail movement. In intact animals, these are evidently under control of the caudal ganglion, linked with outside stimuli via the skin pulse system and the Langerhans axons. Salensky (1903) had inferred that the caudal ganglion must contain sensory as well as motor cell bodies, since he found somata of different sizes in the ganglion; it seems probable that the cells of origin of the Langerhans axons lie in the caudal ganglion, but most of the cells in the ganglion must be motor or associative. Our preliminary ultrastructural observations of the ganglion and caudal nerve cord have shown that there are both chemical synapses and what are presumably electrical junctions within the system, but consideration of the interconnection of cells will be deferred to a later account. The patterns of activity of the tail either involve short bursts of repetitive oscillation at regular intervals, or much longer periods of tail beating upon which a larger rhythm is superposed. This larger rhythm is apparently similar to that produced by surgery or by the action of drugs, when it appears in isolation. We may conclude that at least two types of repetitively firing cells are present in the caudal ganglion, and that they show different sensitivity to blocking agents.

In the lower chordates, such as the dogfish (Gray and Sand, 1936), where a regular swimming pattern is observed in spinal preparations after destruction of the brain, proprioceptive input is required to maintain the motor activity, which is, however, at constant frequency. Our observations have not enabled us to determine whether proprioceptive input is required for rhythmic activity by the caudal ganglion; certainly, the absence of movement in portions of tails lacking the caudal ganglion suggests that local reflexes involving segmental neuron patterns (of the type known in lower chordates and in amphioxus) are absent. Within the caudal nerve itself, there are cell bodies of two sizes, the larger giving rise to axons terminating in corymbiform motor endplates, the smaller to the branching beaded terminations (Fig. 2); there do not appear to be any internuncial or associative

neuron cell bodies, as there are in the segmental neuron patterns of the lower chordate spinal cord. Histological evidence for the existence of sensory termina- tions associated with the caudal muscle cells is unconvincing; our ultrastructural observations suggest that both types of endings mentioned above are motor.

In *O. dioica* swimming bursts of symmetrical oscillation of isolated tails occa- sionally terminate with several tail beats to one side only; such an asymmetry does not suggest that a simple proprioceptive input maintains the usual oscillatory swimming pattern.

In some ways, the tail of *Oikopleura* offers one of the simplest systems utlizing the basic chordate characters of dorsal nervous system, segmental muscle blocks, and notochord to give oscillatory swimming movements but this simple system is different in several ways to that adopted by amphioxus and fishes. In the lower chordates, the nervous system is segmental in arrangement, linked patterns of seg- mental neurons control the oscillatory activity of the myotomes, and are dependent upon proprioceptive input. There are in all lower chordates, from amphioxus upwards, two basic types of muscle fiber in the myotomes, each utilized during different swimming patterns (Bone, 1966), and finally, so far as is known, bursts of oscillatory swimming controlled by higher centers are not observed. It is re- markable that with a small neuron number (some 40 cells in the caudal ganglion, and perhaps 50–60 in the caudal nerve), with only one type of muscle cell, and apparently without proprioceptive information, *Oikopleura* is able to oscillate its tail at different frequencies either continuously or in bursts.

Summary

The skin covering the tail and hinder trunk region of *Oikopleura* propagates impulses at 15–21 cm/sec. Spread is non-decremental and unpolarized. The impulses are of short duration (8–12 msec) and in general resemble skin impulses in hydromedusae more than those of amphibian and tunicate tadpole larvae.

The skin was examined by optical and electron microscopy. The cells are connected by gap junctions. Impulses are assumed to spread by direct current flow from cell to cell.

Electromyograms of tail activity during three behavior patterns (pumping, swimming, and house rudiment expansion) are analyzed in relation to neuromus- cular histology. There appear to be at least two classes of pacemakers, both located in the caudal ganglion. There is no evidence that proprioceptive feedback is required for maintenance of rhythmic activity; and isolation of the tail from the trunk, which contains the cerebral ganglion, does not affect rhythmicity. The

system differs fundamentally from the locomotory systems of *Amphioxus* and fishes.

Skin pulses evoke swimming bursts in intact animals or briefly accelerate pre-existing rhythms. The conduction pathway from skin to caudal ganglion is shown to be a pair of nerves running from the latter to a receptor located in the skin at the back of the trunk. No other nerves end in the skin. Cutting these nerves blocks the response.

As in other cases, the conducting epithelium here functions as an extension of the afferent pathway, mediating an escape response following tactile stimulation.

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